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APPARATUS AND METHOD FOR FORMING MATERIALS

Technical Field

This invention relates to an apparatus and method for forming a plurality of filler elements in a composite material from a phase separating or composite forming material.

Background Art

Methods of producing filaments or fibres have been known in the art for a long time. For example, spinning techniques are used to produce fibres from polymer solutions. British patent specification GB-A-441 440 (Ziegler) discloses one technique in which filaments are produced by passing a liquid raw material through a porous porcelain tube. The filaments emerge from the end of the porous porcelain tube in this disclosure. An operative medium is introduced into the porous porcelain tube through the pores of the tube.

There is currently considerable interest in the development of improved processes and apparatus to enable the manufacture of polymer filaments, fibres, ribbons or sheets. It is theoretically possible to obtain materials with high tensile strength and toughness by engineering the orientation of the polymer molecules and the way in which they interact with one another. Strong, tough filaments, fibres or ribbons are useful in their own right for the manufacture, for example, of sutures, threads, cords, ropes, wound or woven materials. They can also be incorporated into a matrix with or without other filler particles to produce tough and resilient composite materials. Sheets whether formed from fibres or ribbons can be stuck together to form tough laminated composites.

Natural silks are fine, lustrous filaments produced by the silk-worm *Bombyx mori* and other invertebrate species. They offer advantages compared with the synthetic polymers currently used for the manufacture of materials. The tensile strength and toughness of the dragline silks of certain spiders can exceed that of Kevlar™, the toughest and strongest man-made fibre. Spider dragline silks also possess high thermal stability. Many silks are also biodegradable and do not persist in the environment. They are recyclable and are produced by a highly efficient low pressure and low temperature process using only water as a solvent. The

natural spinning process is remarkable in that an aqueous solution of protein is concerted into a tough and highly insoluble material.

According to an article by J. Magoshi, Y. Magoshi, M. A. Becker and S. Nakamura entitled "Biospinning (Silk Fiber Formation, Multiple Spinning Mechanisms)" published in Polymeric Materials Encyclopaedia, by the Chemical Rubber Company, it is reported that natural silks are produced by sophisticated spinning techniques which cannot yet be duplicated by man-made spinning technologies. One feature of the natural spinning process which has not so far been duplicated in man-made spinning technologies is the production in silkworm silk and some spider dragline silks of a plurality of extremely fine elongated structures orientated longitudinally within the fibre each one surrounded by a large number of even finer protein nanofibrils. These structures contribute to the hierarchical composite organisation of silks and are thought to enhance its toughness.

US Patent No US-A-5 444 097 (Tkacik), assigned to the Millipore Corporation teaches a method of making porous polymeric structures in which the composition or temperature of a polymer solution is changed such that the polymer solution becomes thermodynamically instable and separates into two phases. One of the phases in then dissolved and the other phase becomes the porous structure.

An example of a yarn with two components is known from US Patent No US-A-5 380 477 (Kent et al.), assigned to BASF Corporation. In this patent, a multicomponent yarn is manufactured by co-spinning two components. The melting or softening points of each of the components differs by at least 5%. The yarns disclosed in this patent may also include further elements with differing properties. For example, the yarns may incorporate carbon fibres.

Polymer blend fibres having an island-in-the-sea structure are known from US Patent No. US-B-6 245 268. In this application, a phase separation structure is obtained by cooling, drawing and teat-treatment of polymer blends. Extrusion or drawing out of the fibres is carried put at a temperature of between 310°C and 330°C. This patent document emphasises that a mechanical load, such as stress, should not be applied to the polymer blend melt when the phase separation occurs so that the phase separation structure is neither stretched nor deformed. Given that proteins denature at temperatures above 60°C, the teachings of US-B-6 245 268 cannot be applied to the creation of phase separation structures in proteins.

It would furthermore be desirable to be able to add fillers or dopants to the proteins in order to be able to change the properties of the composite matter formed.

Summary of the Invention

There is therefore a need to improve the properties of extruded protein material.

These and other objects of the invention are solved by the use of a starting solution to form a plurality of filler elements in a composite material. The starting solution comprises a miscible solution of at least one protein and a least one further compound, the plurality of filler element being formed from the at least one further compound. Using this starting solution, further materials imparting different properties can be incorporated into the extruded proteins. Preferably the filler elements are filler particles, filler fibrils, elongate fluid-filled cavities, or micelles. A miscible solution is a solution of two or more liquids which are mixed together to give a homogenous solution

In one embodiment of the invention, the starting solution contains a mixture of two or more proteins, synthetic protein analogues or synthetic polymers or a mixture of synthetic polymer or polymers and one or more proteins. This allows a combination of properties to be imparted to the extruded material.

Preferably, the starting solution includes a phase separating agent selected from the group of phase separating agents consisting of proteoglycans, glycosaminoglycans, carbohydrates such as trehalose, sucrose, polyols, peptides or proteins rich in serine and or threonine, glycerol and its derivatives, and detergents to promote the phase separation.

These problems are also solved by providing a method of forming a composite material containing a plurality of filler elements which comprises the following steps:

i) a first step of preparing a starting solution containing a miscible solution of at least one protein and at least one further compound; and

ii) a second step of inducing the starting solution to separate into a bulk phase and a minor phase.

In one embodiment of the invention, the starting solution additionally contains a solvent and the second step is initiated by withdrawing the solvent from the starting mixture. The withdrawal of the solvent is carried out by drying the starting mixture, by the addition of a solvent-binding agent or agents, by altering the pH, by altering the temperature, by changing the pressure by adding inorganic salts, by adding other phase separating compound or compounds, or by a combination of two or more of these factors.

Good fibrous materials have been made from a starting solution comprising a mixture of spidroin I and spidroin II or analogues thereof. In one embodiment of the invention proteins taken or extracted from the silk gland of a lepidopteran insect or from an arachnid are used.

Protein fibres made from the composite forming material are produced by flowing this material through a die. The die can have a convergent form with a converging surface along and towards the axis of the die, in which case the minor phase is elongated substantially parallel to the parallel axis of the die. This allows – in contrast to the disclosure of the prior art US Patent No. US-B-6 245 268 - the formation of fine elongated structures in the composite material The die can also have a divergent form, in this latter case, the small droplets are caused to elongate into elongated filler fibrils or elongated fluid filled cavities orientated in curved hoops describing the axes of elongation as the composite material is flowed through the die.

The invention is also solved by a method of extruding a starting material to form a composite material containing a plurality of filler elements with the following steps:

i) a first step of preparing the starting solution;
ii) a second step of inducing the starting solution to separate into a bulk phase and a minor phase; and
iii) a third step of extruding the starting material either prior to or coincident with the second step to form the composite material.

An apparatus for solving the problem stated and for forming a composite material from a starting solution is also provided. The apparatus has a storage compartment for storing the starting mixture, the starting mixture containing a miscible solution of at least one protein and

at least one further compound and a phase separation compartment for separating the at least one protein and the at least one further compound of the starting mixture into a bulk material containing a plurality of filler elements.

The phase separation compartment comprises a compartment for withdrawing a solvent from the starting mixture by drying, by the addition of a solvent-binding agent or agents, by altering the pH of the starting mixture, by changing the pressure, by adding inorganic salts to the starting mixture, by altering the temperature, by changing the pressure, by adding inorganic salts to the starting mixture, by adding to the starting mixture other phase separating compound or compounds or a combination of these.

In another embodiment of the invention, the apparatus comprises a storage compartment for storing the starting mixture, a phase separation compartment for separating the starting mixture into a bulk material containing a plurality of filler elements and an extrusion compartment for extruding the bulk material.

In a further embodiment, a stable phase separated mixture can be formed and stored elsewhere and subsequently extruded, typically through a divergent or convergent die.

Using this apparatus and method, a composite material comprising at least one protein and at least one further compound is formed. The composite material has a bulk phase and a plurality of filler elements and at least one of the bulk phases or the plurality of filler elements comprise a protein. Preferably the composite material comprises two or more proteins or synthetic protein analogues or synthetic polymers or a mixture of synthetic polymer or polymers and one or more proteins.

A mixture of spidroin I and spidroin II or analogues thereof or fibroin proteins or analogues thereof can be used as proteins. In one embodiment of the invention the at least one protein of the composite material comprises a spinning dope extracted from the silk gland of a lepidopteran insect or from an arachnid.

Description of the Drawings

Fig. 1 is a generalised schematic representation of apparatus for the formation of extruded materials from a composite material;

Fig. 2 is a schematic cross-sectional view along the longitudinal axis of a die assembly of the apparatus shown in Figure 1;

Fig. 3 is a schematic perspective view of the die assembly shown in Figure 2;

Fig. 4 shows micrographs of the luminal contents of the proximal part of the middle division of the silk gland of *Bombyx mori* taken with a differential interference microscope using a x10 microplan objective. In Fig. 4 a the fibroin/ water interface is at the top of the micrograph. A clear zone containing no droplets at the interface 10 minutes after the addition of the water. In Fig. 4 b the fibroin/air interface 10 minutes after drying started showing numerous small droplets developing in the fibroin at the interface. Fig 4 c is as figure 1b but taken 30 minutes after drying started. The droplets have increased in diameter while the zone containing them has widened.

Detailed Description of the Invention

The discovery of the way in which spiders and silkworms produce their silks provides a basis for the invention together with experimentation on spider and silkworm natural spinning solutions. It has been found that by making the walls of the or each tubular passage through which the composite material is extruded at least partly permeable or porous, preferably selectively permeable along the length of the tubular passage, which is preferably tapered, it is possible to control properties such as the pH, water content, ionic composition and shear regime of the composite material in different regions of the tubular passage of the die. Furthermore, the porous walls allow the addition of a phase separating agent to the composite material, such as proteoglycans, glycosaminoglycans, carbohydrates such as trehalose, sucrose, polyols, peptides or proteins rich in serine and or threonine, glycerol and its derivatives, and detergents to the composite forming mixture. The temperature and pressure of the composite forming mixture can also be controlled by adding heaters/coolers to the walls and by raising or lowering the pressure in the tubular passage.

In this invention, the composite forming material comprises at least two polymers. The construction of the tubular passage and the other parts of the apparatus through which the composite forming material is extruded enables the phase diagram of the composite forming material to be controlled. This allows one of the phases of the composite forming material (hereafter termed the minor phase) to separate out of the composite forming material to form small droplets within the rest of the solution (hereafter termed the bulk phase). In addition to small droplets, micelles could also be formed within the composite material.

Conveniently the walls defining the tubular passage(s) are surrounded by said enclosure means to provide one or more compartments. These compartments act as jackets around the tubular passage(s). The or each tubular passage suitably has an inlet at one end to receive the composite forming material and an outlet at the other for the formed or extruded composite material and is typically divided into three parts arranged consecutively, the first part or initial zone allowing for the pre-treatment and pre-orientation of the fibre-forming polymer molecules in the liquid feedstock prior to forming the composite material by draw down, the second region or subsequent zone in which draw down of the "thread" takes place and which functions as a treatment and coating bath, and the third part or final part has an outlet or opening of restricted cross-section which serves to prevent the loss of the contents of

the „treatment bath" with the emerging fibre and to provide for the commencement of an optional air drawing stage.

It will be appreciated that any solution or solvent or other phase or phases surrounding the fibre in the second part of the or each tubular passage also serves to lubricate the fibre as it moves through and out of the tubular passage. The solution or the solvent can be removed through the porous or semi-permeable walls of the tubular passage. The removal of the solution or the solvent can also lead to phase separation in the composite forming material.

All or part of the length of each tubular passage typically has a convergent geometry typically with the diameter decreasing in a substantially hyperbolic fashion. According to G. Y. Chen, J.A. Cuculo and P. A. Tucker in an article entitled "Characteristic and Design Procedure of Hyperbolic Dies" in the Journal of Polymer Sciences: Part B: Polymer Physics, Vol, 30, 557-561 in 1992, it is reported that the orientation of molecules in a fibre can be improved by using a die with a convergent hyperbolic geometry instead of the more usual parallel capillary or conical dies.

In another embodiment of the invention, the die has a divergent form. In this case, the small droplets are caused to elongate into elongated filler fibrils or elongated fluid filled cavities orientated in curved hoops describing the axes of elongation as the composite material is flowed through the die

The geometry of substantially all or part of the or each tubular passage may be varied to optimise the rate of elongational flow in the composite material and to vary the cross-sectional shape of the formed material produced from it. The preferred substantially hyperbolic taper for part or all of the or each tubular passage maintains a slow and substantially constant elongational flow rate thus preventing unwanted disorientation of the fibre-forming molecules resulting from variation in the elongational flow rate or from premature formation of insoluble material before the composite forming material has been appropriately reoriented. A convergent taper to the tubular passage of the die will induce elongational flow which will tend to induce a substantially axial alignment in the fibre-forming molecules, short fibres or filler particles contained in the composite forming material by exploiting the well known principle of elongational flow. Alternatively, the principle of elongational flow through a divergent instead of the convergent die can be used to induce

orientation in the hoop direction that is substantially transverse to the direction of flow through the divergent part of the die.

It should be noted at this stage that if micelles are formed in the composite material, these will not be elongated due to their small size.

The diameter of the or each tubular passage may be varied to produce fibres of the desired diameter.

The rheology of the liquid composite forming material in the tubular passage of the die is largely independent of scale, thus enabling the size of the apparatus to be scaled up or down. The convergence of the tubular passage allows a wide range of drawing rates to be used typically ranging from 0.01 to 1000 mm sec^{-1}. It is technologically possible to have higher drawing rates. If fibres are being extruded they may typically have a diameter of from 0.1 to 100 μm. Typically the outlet of the tubular passage has a diameter of from 1 to 500 μm with the diameter of the inlet of the tubular passage being from 25 to 150 times greater depending on the extensional flow it is desired to produce. Tubular passages of alternative cross-sectional shapes can be used to produce fibres, flat ribbons or sheets of extruded materials with other cross-sectional shapes.

All or part or parts of the walls of the or each tubular passage of the die assembly are constructed from or formed or moulded from selectively permeable and/or porous material, such as cellulose acetate-based membrane sheets. The membrane can be substituted with diethylaminoethyl or carboxyl or carboxymethyl groups to help maintain protein-containing composite forming materials in a state suitable for spinning. The membrane can be rendered substantially hydrophobic with a siliconizing or silanizing solution or with polytetrafluoroethylene particles. Other examples of permeable and/or porous material are hollow-fibre membranes, such as hollow fibres constructed from polysulfone, polyethyleneoxide-polysulfone blends, silicone or polyacrylonitrile. The exclusion limit selected for the semipermeable membrane will depend on the size of the small molecular weight constituents of the composite forming material but is typically less than 12 kDa.

All or part of the walls of the or each tubular passage can be constructed from selectively permeable and/or porous material in a number of different ways. By way of

example only a selectively permeable and/or porous sheet can be held in place over a groove with suitable geometry cut into a piece of material to form the tubular passage. Alternatively two sheets of selectively permeable and/or porous material can be held in place on either side of a separator to construct the tubular passage. Alternatively a single sheet can be bent round to form a tubular passage. A hollow tube of selectively permeable and/or porous material can also be used to construct all or part of the tubular passage. By way of example only, a variety of methods are available to shape the tube into a die as is commonly known to a craftsman skilled in the art.

The interior walls may furthermore be substantially smooth or may be provided with "ridges" or bumps on at least part of the wall. The presence of such modifications in the walls aids in the draw-down process.

The use of selectively permeable and/or porous walls of substantially all or part or parts of the tubular passage(s) enables the proper control within desired limits of, for example, the concentration of fibre-forming material; solute composition; ionic composition; pH; dielectric properties; osmotic potential and other physico-chemical properties of the composite forming material within the tubular passage by applying the well-known principles of dialysis, reverse dialysis, ultra-filtration and pre-evaporation. Electro-osmosis can also be used to control the composition of the composite forming material within the tubular passage. It will be appreciated that a control mechanism receiving inputs relating to the product being formed, for example the diameter of the extruded composite material and/or the resistance countered in the tubular passage, such as during extrusion through the outlet of the tubular passage, can be used to control, for example, polymer concentration, solute composition, ionic composition, pH, dielectric properties, osmotic potential and/or other physicochemical properties of the composite forming material within the tubular passage.

The selective permeability and/or porosity of the walls of the or each tubular passage may also allow for the diffusion through the walls of further substances into the tubular passage(s) provided that these have a molecular weight lower than the exclusion limit of the selectively permeable material from which the walls of the tubular passage(s) are constructed. By way of example only the additional substances added to the composite forming material in this manner may include surfactants; dopants; coating agents; cross-linking agents; hardeners;

and plasticizers. Larger sized aggregates can be passed through the walls of the tubular passage if it is porous rather than being simply semipermeable.

The compartments surrounding the walls of the tubular passage or passages may act as one or more treatment zones or baths for conditioning the fibre as it passes through the tubular passage(s). Additional treatment can occur after the material has exited the outlet of the tubular passage.

One or more regions of the or each tubular passage may be surrounded by one or more compartments arranged consecutively so as to act as a jacket or jackets to hold solution, solvent, gas or vapour in contact with the outer surface of the selectively permeable walls of the tubular passage(s). Typically solution, solvent, gas or vapour is circulated through the compartment or compartments. The walls of the compartment or compartments are sealed to the outer surface of the wall or walls of the tubular passage(s) by methods that will be understood by a person skilled in the art. The compartment or compartments serve to control the chemical and physical conditions within the or each tubular passage. Thus the compartments surrounding the tubular passage(s) serve to define the correct processing conditions within the composite forming material at any point along the tubular passage(s). In this way parameters such as the temperature; hydrostatic pressure; concentration of fibre-forming material; pH; solute; ionic composition; dielectric constant; osmolarity or other physical or chemical parameter can be controlled in different regions of the tubular passage as the composite forming material moves down the length of the die. By way of example only, continuously graded or stepped changes in the processing environment can be obtained.

Conveniently a selectively permeable/porous membrane can be used to treat one side of a forming extrusion in a different way to the other side. This can be used, for example, to coat the extrusion or remove solvent from it asymmetrically in such a way that the extrusion can be made to curl or twist.

All or part of the draw down process may typically occur within the tubular passage of the die rather than at the outer face of the die assembly as occurs in existing spinning apparatus. The former arrangement offers advantage over existing spinning apparatus. The distortion of molecular alignment due to die swell is avoided. The region of the die assembly after the internal commencement of the draw down taper can be used to apply coatings or

treatments to the extrusion. Further, the last part of the die assembly is water lubricated by the solvent-rich phase surrounding the extrusion.

By way of example only the apparatus can be used for forming fibres from composite forming materials initially containing miscible or homogenous solutions of recombinant spider silk proteins or analogues or recombinant silk worm silk proteins or analogues or mixtures of such proteins or protein analogues or regenerated silk solution from silkworm silk. When these composite forming materials are used it is necessary to store the composite forming material at a pH above a critical value to prevent the premature formation of insoluble material. It will be appreciated that other constituents may be added to the composite forming material to prevent premature solidification or gelling of the proteins or protein analogues in solution. These constituents may then be removed through the semipermeable and/or porous walls when the composite forming material has reached the appropriate portion of the tubular passage in which it is desired to induce the transition from liquid composite forming material to solid composite material, e.g. thread or fibre. The composite forming material within the tubular passage can then be brought by dialysis against an appropriate acid or base or buffer solution to a pH value at or close to the critical value to induce aggregation or conformation change in one or more of the constituent proteins of the composite forming material. Such a pH change will promote the formation of an insoluble material. A volatile base or acid or buffer can also be diffused through the walls of the or each tubular passage from a vapour phase in the surrounding compartment or jacket to adjust the pH of the composite forming material to the desired value. Vapour phase treatment to adjust the pH can also occur after the extruded material has left the outlet of the die assembly.

The draw rate and length, wall thickness, geometry and material composition of the or each tubular passage may be varied along its length to provide different retention times and treatment conditions to optimise the process.

One or more regions of the walls defining the or each tubular passage can be made impermeable by coating their inner or outer surfaces with a suitable material to modify the internal environment in a length of the tubular passage using any coating method as will be understood by a person skilled in the art.

The inner surface of the walls of the or each tubular passage can be coated with suitable materials to reduce the friction between the walls of the tubular passage and the

composite forming material or fibre. Such a coating can also be used to induce appropriate interfacial molecular alignment at the walls of the tubular passage in liquid crystalline polymers when these are included in the composite forming material.

In this application, a composite forming material is disclosed which is prepared from a phase separating mixture containing two or more miscible components which, for example, may be different proteins. The removal or addition of components through the selectively permeable and/or porous material can be used to control the phase separation process to produce droplets of one or more components typically with a diameter of 100 to 5000 nm within the bulk phase in the final extrusion. These can be used to enhance the toughness and other mechanical properties of the extrusion. The use of a convergent or divergent die conveniently induces elongational flow in the droplets to produce orientated and elongated filler particles or voids within the bulk phase. A convergent die will orientate and elongate such droplets in a direction parallel to that of the formed product whereas a divergent die will tend to orientate the droplets in hoops transverse to the direction of flow of each particle within the tubular passage of the composite forming material. Both types of arrangement can be used to enhance the properties of the formed composite material. Further it will be understood that the selectively permeable and/or porous walls of the or each tubular passage can be used to diffuse in or out chemicals to initiate the polymerisation of filler particles.

The spinning apparatus with one or more tubular passages surrounded by a compartment or compartments to act as jackets can be constructed by one or two stage moulding or other methods known to a person skilled in the art. It will be appreciated that a moulding process can be used to create simple or complex profiles for the or each tubular passage and the outlet of the die assembly. Very small flexible lips can be formed, e.g. moulded, at the outlet to prevent the escape of the contents of the treatment bath and act as a restriction to enable an optional additional air drawing stage or wet drawing after the material has left the outlet of the die assembly should this be required. The microscopic profile of the inner surface of the lips at the outlet can be used to modify the texture of the surface coating of the extruded material.

By way of example only, the jackets and supports for the tubular passages can be constructed from two or more components formed by injection moulding or constructed in other ways as will be understood by a person skilled in the arts. It will be appreciated that this

method of construction is modular and that a number of such modules can be assembled in parallel to produce simultaneously a number of fibres or other shaped products. Sheet materials can be produced by a row or rows of such modules. Such a modular arrangement allows for the use of manifolds to supply composite forming material to the inlet of the tubular passage(s) and to supply and remove processing solvents, solutions, gases or vapours to and from the jacket or jackets surrounding the tubular passages. Additional components may be added if desired. Potential modifications to the arrangements shown will be apparent to persons skilled in the art.

Other methods of constructing spinning apparatus in which the walls of the tubular passages are substantially or partially constructed from semipermeable and/or porous material or materials will be known by a person skilled in the art. By way of example only these include micro-machining techniques, laser ablation techniques and lithography techniques. In addition it will be appreciated that walls of the tubular passages substantially or partially constructed from semipermeable/porous material can be incorporated into other types of spinning apparatus, such as electrospinning apparatus.

The or each tubular passage may be made self-starting and self-cleaning. It will be appreciated that blockage of spinning dies during the commercial production of extruded materials is time-consuming and costly. To overcome this difficulty, the walls of the tubular passage may be constructed by two or more jackets arranged in sequence. The pressure in each of these jackets can be varied independently by methods that will be understood by a craftsman skilled in the art. Pressure changes in the jackets can be used to change the diameter of different regions of the tubular passage in a manner analogous to a peristaltic pump to pump the composite forming material to the outlet to commence the drawing of fibres, to clear a blockage or to induce phase separation. Thus a decrease in pressure in a jacket towards the outlet end of the tubular passage will dilate the elastic walls of the tubular passage within the jacket. If the pressure is now raised in a second jacket closer to the input end of the tubular passage a region of the walls of the tubular passage running through this jacket will tend to collapse forcing the composite forming material towards the outlet. Alternatively, the pressure in the composite forming material fed to the tubular passage could be increased causing the diameter of the elastic tubular passage walls to increase. It will be appreciated that both methods could be used together or consecutively. With both methods, the elasticity of the passage walls enables the diameter of the tubular passage to be increased reducing the

resistance to flow. With both methods it is to be noted that increasing the pressure of the composite forming material will also assist in start up and in clearing blockages in the tubular passage. It will also be appreciated by way of example only that the use of rollers such as are used in peristaltic pumps can be used as an alternative means of applying pressure to pump composite forming material to the outlet to commence spinning or to clear a blockage.

The apparatus my be arranged in such a way that two or more fibres are formed in parallel and twisted around each other or crimped or wound onto a former or coated or left uncoated as desired. The fibres can be drawn through a coating bath and subsequently through a convergent die to give rise to an "island-in-the-sea" composite material as will be understood by a person skilled in the art. One or more rows of dies or one or more dies with slit or annular opening can be used to form sheet materials.

Best Mode for Carrying out the Invention

Figure 1 shows a schematic apparatus for the formation of composite materials from a composite forming material or a dope 25 comprising at least two components. At least one of the components of the composite forming material is a protein, such as a spidroin or fibroin protein. The other one of the components of the composite forming material can be a protein or can be a dopant to be included in the protein. The at least two components form a homogenous solution. The composite forming material 25 may also include phase separating agents that aid in the phase separation of the components of the composite forming material 25. Such phase separating components include, but are not limited to proteoglycans, glycosaminoglycans, carbohydrates (such as trehalose or sucrose), polyols, peptides or proteins rich in serine and or threonine, glycerol and its derivatives, and detergents

The apparatus comprises a dope reservoir 1 containing the composite forming material 25; a pressure regulating valve or pump means 2 which maintains a constant output pressure under normal operating conditions; a connecting pipe 3; and a spinning die assembly 4 comprising at least one spinning tube or die further described in figures 2 to 5. A take-up drum 5 of any known construction draws out and reels up extruded material at a constant tension exiting from the outlet of the die assembly 3. The pressure regulating valve or pump

means 2 may be any device normally producing a constant pressure commonly known to a person skilled in the art.

The arrangement shown in Figure 1 is purely exemplary and additional components to the arrangement shown in Figure 1 will be apparent to persons skilled in the art. In use, the composite forming material 25 is passed from the feedstock reservoir 1 at a constant low pressure by means of the regulating valve or pump means 2 via the connecting pipe 3 to the inlet of the spinning die assembly 4.

The die assembly 4 is shown in greater detail in Figures 2 and 3 and comprises a first spinning tube or die 8 upstream of a second spinning tube or die 12, the dies together defining a tubular passage 17 for composite material 25 through the die assembly 4. The die 12 has an interior wall 18 and is divided into an initial zone 60 and a subsequent zone 62. The dies 8 and 12 are made of semipermeable and/or porous material, such as cellulose acetate membranes or sheets. Other examples of suitable semipermeable and/or porous materials are diethylaminoethyl or carboxyl or carboxymethyl groups which help to maintain protein-containing dopes in a state suitable for spinning. Hollow-fibre membranes material, such hollow-fibre membranes being made from polysulfone, polythyleneoxide-polysulfone blends, silicone or polyacrylonitrile can also be used. The exclusion limit selected for the semipermeable membrane will depend on the size of the small molecular weight constituents of the spinning dope 25 but is typically less than 12 kDa.

The die 8 is held at its upstream end by a tapered adaptor 6 positioned at the inlet end of the die assembly 4 and at its downstream end by a tapered adaptor 7 positioned internally in the die assembly 4. The die 8 is held at its upstream end by the adaptor 7 and at its downstream end by a spigot 13 at the outlet of the die assembly 4. The die 8 has a convergent, preferably hyperbolic, internal passage and the geometrical taper is preferably continued with the internal passage of the die 12. This can be achieved during construction by softening a semipermeable tube or die an a warmed suitably tapered mandrel, or by other methods as will be appreciated by a craftsman skilled in the art before fitting the spinning tube or die into the apparatus. The internal passages of the dies 8 and 12 together provide the tubular passage 17 for composite material from the inlet to the outlet of the die assembly 4.

A jacket 9 surrounds the die 8 and may contain a fluid, e.g. a solvent, solution, gas or vapour to control the processing conditions within the spinning tube or die 8. The jacket 9 is fitted with an inlet 10 and an outlet 11 to control flow of fluid into and out of the jacket. A further jacket 14 surrounds the tube or die 12 and is fitted with a fluid inlet 15 and a fluid outlet 16 to enable fluid, e.g. solvent, solution or gas, to be passed into and out of the jacket 14 in contact with the semipermeable/porous walls of the die 12.

As an alternative to the die 8 shown having semipermeable walls, a die 8 may be constructed from material which is not semipermeable or porous but which is preferably tapered, e.g. convergently, and may be temperature-controlled by circulation fluid at a predetermined temperature through the jacket 9.

In operation, the composite forming material 25 is fed to the inlet of the die 8, as the composite forming material 25 passes along the tubular passage 17 it is treated firstly as it passes through the die 8 and secondly as it passes through the die 12. The fluid passing through the jacket 9 may merely serve to heat or maintain the composite forming material 25 at the correct temperature or provide the correct external pressure to the walls of the die 8. In this case it is not essential for the walls of the die to be made of semipermeable and/or material. The temperature of the dies 8 and 12 for the extrusion of the composite forming material 25 should typically be maintained at a temperature of about 20°C but spinning may be carried out at temperatures as low as 2°C and as high as 40°C. It should be noted that spinning of materials containing proteins should not be carried out at temperatures higher than 40°C as there is a risk that the proteins denature at these temperatures. The temperature of the dies 8 and 12 for the extrusion of dopes can more generally be as high as 100°C providing that the material is not destroyed at this temperature. The pressure of the fluid, liquid or gas, in the jackets surrounding the walls of the tubular passage 17 is typically maintained at a pressure close to that at which the composite forming material 25 is supplied to the die assembly 4. However the pressure can be somewhat higher or lower depending on the geometry of the dies and the strength of the generally flexible semipermeable and/or porous membrane. "Chemical" treatment of the composite forming material 25 occurs during "draw down" as the composite forming material 25 passes through the die 12 although chemical treatment may also occur as the composite forming material 25 passes through the die 8 if the walls of the latter are at least partly made of semipermeable material. In Figures 2 and 3, the abrupt pulling away of the composite forming material 25 from the walls of the die 12 at 12A

indicates the internal draw down of the "fibre". This occurs at the boundary of the initial zone 60 and the subsequent zone 62.

Within the tubular passage 17, the phases of the composite forming material 25 separate out into their individual components due to the "chemical" treatment of the composite forming material 25 or changes in the physical environment of the composite forming material 25. In general two phases are formed, although it is possible for more than two phases to be formed. The first phase – or the bulk phase – has the majority of the component and acts as a matrix. The second phase – or the minor phase – is incorporated into the bulk phase in the form of small droplets or micelles. The small droplets may be either fluid-filled or can be solid filler particles. In one embodiment of the invention, the minor phase includes a material which can be cross-linked to form either hard or elastomeric filler elements. Examples of such materials include concentrated protein solutions, spidroin I or II or fibroin,

The pulling away of the "fibre" from the die walls at 12A occurs at a place in the tubular die 12 where the force required to produce extensional flow to create a new surface just falls below the force required to flow the composite forming material through the die 12 in contact with the die walls. This is the position at which the surface energy of the interior wall 18 becomes lower than the surface energy of the composite forming material 25. The position of 12A will depend on: the changing rheological properties of the composite forming material; the rate and force of drawing; the surface properties of the die 12; the surface properties of the lining of the die 12; and the properties of the composite forming material and the aqueous phase surrounding the composite forming material.

It will be appreciated that the temperature, pH, osmotic potential, colloid osmotic potential, solute composition, ionic composition, hydrostatic pressure or other physical or chemical factors of the solution, solvent gas or vapour supplied to the jacket(s) control or regulate the conditions inside the tubular passage 17 as is commonly understood by a craftsman skilled in the art. Chemicals in the fluid supplied to the jacket(s) 9 are able to pass through the semipermeable and /or porous walls of the tubular passage 17 to "treat" the composite forming material 25 passing therethrough. It is also possible for chemicals in the composite forming material 25 to pass outwardly through the semipermeable and/or porous walls of the tubular passage 17. The fluids supplied to the composite forming material 17 will

obviously depend on the type of composite forming material 25 used and the semipermeable and/or porous membranes used. However, by way of example only, for the spinning of concentrated spider major ampullate gland protein solutions, the jacket 9 may contain 100 mM Tris or PIPES buffer solution, typically at a pH of 7.4, and 400 mM sodium chloride to help maintain the folded state of the protein. The jacket 14 may contain 100 mM ammonium acetate buffer solution at al lower pH, typically <5.0, and 250 mM potassium chloride to encourage the unfolding /refolding of the protein. High molecular weight polyethylene glycol can be added to the solution in both jackets to maintain or reduce the concentration of water in the composite forming material 25.

It will be realised that the spinning tube or die 12 can be folded back on itself or coiled or arranged in other ways between the tapered collar 7 and the spigot 13. The diameter and cross-sectional shape or the exit 13 can be varied or adjusted to suit the diameter and cross sectional shape of the formed material. For a formed product having a circular cross-sectional, the typical diameter of the outlet is from 1 to 100 μm and the typical diameter of the inlet to the tubular passage 17 would be from 25 to 150 times greater than the outlet diameter depending on the extent of the extensional flow. It will be appreciated that the arrangements and proportions shown in Figure 2 are purely exemplary and thus that additionally components may be added if desired. Potential modifications to the arrangements shown in Figure 2 will be apparent to persons skilled in the art.

The permeability or porosity of the walls of the tubular passage may be the same throughout the length of the latter. Alternatively, however, if the tubular passage 17 passes through more than one treatment zone the permeability/porosity of the walls of the tubular passage may change from treatment zone to treatment zone by using different semipermeable or porous materials for the walls of the tubular passage. Thus the walls of the tubular passage 17 may comprise: semipermeable material of the same permeability throughout the length of the tubular passage; semipermeable material of different permeability for different portions of the tubular passage; porous material of the same porosity throughout the length of the tubular passage 17; porous material of different porosity for different portions of the passage; or semipermeable material for one or more portions of the length of the tubular passage and porous material for one or more other portions of the tubular passage. As mentioned above, some portions of the walls of the tubular passage may be non-permeable. By way of example only, suitable semipermeable materials are: cellulose derivatives, expanded PTFE,

polysulfone, polyethylenoxide-polysulfone blends, and silicone polyacrylonitrile blends. By way of example only, the suitable porous materials are: polyacrylate, poly (lactide-co-glycolide), porous PTFE, porous silicon, porous polyethylene, cellulose derivatives and chitosan.

It will be appreciated that the apparatus is suitable for the information of fibres of sheets from all solutions of lyotropic liquid crystal polymers whether synthetic or man-made or natural or modified or copolymer mixtures or solutions of recombinant proteins or analogues derived from them or mixtures of these. By way of example only these include collagens; certain cellulose derivatives; spidroins; fibroins; recombinant protein analogues based on spidroins, or fibroins, and poly (p-phenylene terephthalaes). The method is also suitable for use with other polymers or polymer mixtures provided that they are dissolved in solvents, whether aqueous or non-aqueous, protein solutions, cellulose or chitin solutions. It will also be appreciated that the use of one or more semipermeable and/or porous treatment zones can be used for dies or die assemblies having essentially annular or elongated slit openings used for the formation of sheet materials.

Examples

Example 1

Highly concentrated aqueous fibroin was prepared as follows. Early final instar *Bombyx mori* silkworm larvae were selected by their size and light colour, lacking the darker pigmentation seen in late final instar larvae immediately before cocoon spinning. This stage was selected because the fibroin in the middle division of the silk gland was still present as a highly viscous sol, having yet to reach the gelled state seen in late final instar larvae. Similar preliminary results were obtained using protein removed from the proximal zone (A-zone) of the major ampullate silk gland of the spider *Nephila edulis* but the much smaller size of the gland and smaller number of spiders available precluded detailed investigation.

The selected silkworm larvae were opened and the silk glands removed and placed for approximately five minutes in distilled water in a plastic dish to facilitate removal of the epithelium from the middle division. The proximal part of the middle division was selected as this contains much less sericin than the distal part. This is because layers of sericin are

progressively secreted around the fibroin core by the epithelium as the fibroin core moves through the middle division. A blob of viscous protein taken from the proximal part of the middle division was washed for 10 minute in a large excess of distilled water with occasional very gentle agitation to remove the thin layer of sericin coating. Thereafter the still coherent lump of fibroin was gently lifted from the dish with watchmaker's forceps and blotted with filter paper for 10 sec to remove excess water. The blob of fibroin was allowed to spread for 5 to 10 minutes on a microscope slide.

Examination with the differential interference microscope of the blotted fibroin at this stage under a dry cover slip showed that it contained two phases, a clear bulk phase and numerous small spherical droplets ranging from 0.5 to 2 μm in diameter. These droplets were fairly uniformly distributed throughout the bulk phase and extending right up to the air / fibroin interface. The droplets were also seen in material taken from the middle division immediately after opening the larva and are therefore not produced by ageing of the fibroin. When the air surrounding the spread fibroin blob was replaced with distilled water by allowing it to flow under the edge of the cover slip, a different appearance developed at the sharp interface between water and fibroin. The droplets in the bulk phase disappeared from the fibroin close to the distilled water leaving a single clear phase (Figure 4 a).

Observation of individual droplets showed that they could dissolve quite rapidly into the bulk phase sometimes shrinking and disappearing within five seconds to produce a single phase. This left a zone free of droplets that widened with time, spreading inwards from the fibroin/water interface. This is produced by the inward diffusion of water into the fibroin blob. The rate of widening of the clear zone decreased progressively with time presumably because the rate of diffusion of the water into the fibroin slowed with increasing distance from the fibroin/water interface.

These observations indicate that the concentrated nascent silk is stored in the middle division of the gland as a two phase system and that these two phases unite to give a single phase when the protein is slowly diluted.

Example 2

The origin and fate of the small droplets in the secretory pathway was studied in whole mounts of the *Bombyx* silk glands and duct. These were prepared by infusing a buffered fixative solution containing glutaraldehyde and formaldehyde into the abdominal cavity of freshly opened final instar silkworm larvae in the act of spinning silk. The formaldehyde was freshly prepared by depolymerizing paraformaldehyde.

After fixation for one hour the excised gland and duct was mounted whole in Farant's gum and allowed to clear before examination. In some cases transverse sections of the middle division were prepared using a simple pith microtome and examined after clearing in Farant's gum. The fibroin in the posterior and middle division of the gland was seen to contain small droplets approximately 0.5 to 2 μm in diameter. These droplets appeared to elongate progressively as the nascent silk fibroin passed down through the converging lumen of the anterior division and duct. A similar effect has been observed in the secretory pathway of the major ampullate silk gland of the spider *Nephila edulis* as reported by Knight, D. P. and F. Vollrath in "Liquid crystals and flow elongation in a spider's silk production line." <u>Proceedings of the Royal Society of London Series B-Biological Sciences</u> **266**(1418): 519-523.

Whole mounts of the A-zone of the major ampullate gland of the spider *Nephila edulis* were also examined in the present study. The much smaller diameter of the ampulla compared with of the middle enabled more detailed observation with the differential interference microscope. A narrow clear zone was observed in the A-zone luminal contents immediately adjacent to the epithelium. Central to this the packing density and size of the spherical droplets increased with distance from the luminal surface. This suggests that the luminal contents are initially secreted as a single phase that in time undergoes phase separation into bulk and droplet phases. To demonstrate that shearing could deform the small droplets, concentrated spider and silkworm nascent silk taken from the proximal part of the middle division of the silkworm gland or A-zone of the spider silk gland was mounted between slide and cover slip and gently sheared by displacing the cover slip approximately 5 mm. The small droplets were seen to have elongated in the direction of shear. Similar effects were observed in droplets subjected to divergent flow produced by allowing concentrated nascent dope was to flow out of the cut end of spider and silkworm glands mounted in distilled water. In this case the droplets elongated in the hoop direction parallel to the axes of elongation.

These observations suggest that the droplets in *Bombyx mori* persist throughout the secretory pathway from the posterior division through to the final silk thread. It also suggests that they elongate by elongational flow in the convergent duct and by extensional flow in the draw down taper to give rise to extremely narrow elongated structures running parallel to the long axis of the silk brins in the Transmission Electron Microscope. They also demonstrate that elongation of the droplets and orientation of the axis of elongation can be manipulated by strain produced by elongational or extensional flow and by shear.

Example 3

To study the formation of droplets from a concentrated fibroin solution, a blob of the blotted contents of the proximal middle division of the gland was prepared as described above. This was transferred to a tared Eppendorf 1.5 ml centrifuge and reweighed. 1 μl of distilled water was added per mg of blotted dope and the tube gently very gently agitated in a water bath at 4°C overnight and then allowed to stand for half an hour to sediment any cell debris. Centrifugation was not used as the solution is very sensitive to shear.

Drops of the resulting concentrated supernatant solution were completely clear when examined in the differential interference microscope without drying. The solution was then allowed to dry slowly between slide and cover slip under the microscope. A narrow zone containing many small droplets 0.5 -15 μm in diameter formed at the interface between solution and air at the edge of the cover slip within 10 minutes (Figure 4 b). This zone widened as the solution dried and the droplets increased in size to 5-40μm within 30 minutes (Figure 4c). The number and size of the droplets varied in a graded fashion across the zone with a high density of small droplets adjacent to the solution/water interface and fewer larger droplets at a somewhat greater distance from it (Figure 4c). The larger size of the latter droplets may result from more rapid growth caused by less competition from other nucleation sites. The packing density of the droplets immediately adjacent to the interface increased progressively with time and within 1 to 3 hours they appeared to have coalesced to form to form a narrow zone of continuous phase. Occasional spherulitic crystals, resembling those of fibroin described by Magoshi et al. in an article "Crystallization of silk fibroin from solution." Thermochimica Acta 352: 165-169 (2000) grew at the outermost edge of this suggesting a direct transition from coalesced continuous phase to a crystalline phase.

These observations show that a phase separation develops as concentrated fibroin solutions dry.

In summary, the observations described in connection with the above examples show that the phase separations can be manipulated in the nascent silk solutions taken from silkworms and spiders by controlling the water content. They also show that small droplets forming a dispersed phase within the concentrated nascent silk solutions can be extended and orientated by extensional flow and shear.